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(54) Title: A PROCESS FOR PRODUCING AND PACKAGING LACTIC ACID BACTERIA TABLETS

(57) Abstract: A process for producing lactic acid bacteria tablets wherein a mass is formed by mixing lactic acid bacteria and carrier, said mass comprising lactic acid bacteria approximately 7 x 10^9 CFU/g. The tablets are formed from said mass under a pressure that enables a substantial amount of the bacteria to remain viable but makes the tablets hard enough to withstand pressing through the aluminium blister of the package. The tablets are packaged in aluminium blisters directly after the formation of the tablets.

A PROCESS FOR PRODUCING AND PACKAGING LACTIC ACID BACTERIA TABLETS

The present invention relates to a process for producing and packaging lactic acid bacteria using a method where the bacteria stays viable during the tabletting and storing and the tablets are packaged properly for storage and transportation without damages.

The group of lactic acid bacteria (LAB) is genetically very divergent. With occasional exceptions, the lactic acid bacteria are aerotolerant anaerobes which means that they possess the fermentative type of metabolism associated with anaerobes and are also indifferent to the presence of oxygen. Many species of different lactic acid bacteria genera are of paramount importance in the food industry, both as beneficial organisms and as spoilage organisms. They are widely used as starter strains for manufacturing cheese, yoghurt, sour bread and other fermented food products (vegetables, fish, sausages), as well as for prevention of spoilage of silage. In addition, several health benefiting (probiotic) effects have been reported for lactic acid bacteria (especially lactobacilli) colonizing the gastrointestinal tract; e.g. stimulation of immunoglobulin production, induction of interferon expression in macrophages, acidification of the local environment, production of H_2O_2, hypocholesteraemic effects, binding of mutagenic compounds, production of bacteriocins and prevention of adherence of pathogenic bacteria like *Salmonella typhimurium* and *Neisseria gonorrhoeae* to the epithelial cell. Administration of lactic acid bacteria (e.g. *Enterococcous faecium*) or fermented products thereof is effective in promoting growth and curing diseases like diarrhea, caused by e.g. *Eschericia coli*.

It is known that to provide a reasonable physiological effect, lactic acid bacteria must be administered at a minimum dose of about 1×10^9 CFU/g, preferably 3×10^9 CFU/g. Lactic acid bacteria are generally administered in a powder form, either in sachets, in capsules or alternatively in a liquid form, yoghurt being the most used liquid form. Lactic acid bacteria have not been traditionally provided in tablet form

A problem encountered in the attempts to produce lactic acid bacteria tablets has been to keep the bacteria viable through the process. When tablets containing micro-organisms are made in conventional processes, the living micro-organism is usually dried, reduced to

powder, added to a basic compounding material for forming the tablet and then molded into a tablet. In the conventional process it is difficult to make tablets containing mainly viable lactic acid bacteria and only little other substances, e.g. carrier just enough for enabling tablet formation.

Lactic acid bacteria cannot survive the hard pressure and the high temperature that are needed in forming conventional tablets. High pressure must be used to provide tablets having a proper structural strength to maintain the integrity during normal conditions of production, packaging, shipping and handling. The high pressure used in conventional processes causes a loss of a considerable amount of the lactic acid bacteria. Thus the amount of viable bacteria in the final product is quite low. If low pressure is used the tablets have a soft structure that does not withstand transportation and packaging e.g. in cans. If the tablets are packaged in aluminium blisters, high temperature is needed during packaging, which is harmful to the viable organisms.

EP 0955058 discloses a process for making very hard tablets containing lactoferrin and lactulose as active ingredients. Lactulose is an important ingredient in the invention according to this patent since it makes it possible to form hard tablets without too much pressure. Tablets containing lactoferrin and lactic acid bacteria were also made in the process according to JP 1221319. However, tablets containing purely lactic acid bacteria and carrier were not made. In EP 0599479 tablets were made on powdered *Lactobacillus brevis* subsp. *coagulans* comprising $2 \times 10^9 - 5 \times 10^{10}$ bacteria / g. When preparing the tablets the powdered bacteria was adjusted to contain $10^6 - 10^8$ bacteria / g. The tablet size was 250 mg and six tablets had to be administered daily to provide 3×10^8 bacteria / day. In US 4,396,631 confectionery tablets containing about 2×10^8 / g anaerobic bifidobacteria were made.

The amount of viable lactic acid bacteria in the final product remains higher when the product is in liquid form. Storing and transporting liquid form lactic acid bacteria can, however, cause problems, e.g. due to mold growth, since lactic acid bacteria mold easily if the humidity of the product is too high. Liquid form lactic acid bacteria products cannot be transported long distances without costly transportation systems.

Lactic acid bacteria tablets would, thus, be the most convenient form of lactic acid bacteria containing products since they would have many advantageous properties compared to liquid products. Tablets can easily be stored for rather long periods and transported without damages and no special equipment or conditions are needed. Tablets can be easily administered orally as one dosing unit.

Transporting and packaging requires a certain hardness of the tablets. However, tablets cannot be made very hard since lactic acid bacteria cannot survive the pressure that is needed to form hard tablets. It has been impossible to produce lactic acid bacteria tablets containing large amounts of viable bacteria. In the prior art only tablets containing physiologically insufficient amounts of lactic acid bacteria as one of the active ingredients have been produced.

For producing lactic acid bacteria tablets containing a physiologically sufficient amount of viable bacteria a special new technique is needed since the conventional tabletting systems require a higher temperature than the bacteria can survive (max 45 °C) and as well as a high pressure. The combination of high temperature and high pressure results in a low amount of viable bacteria already during the production process.

Packaging of the tablets is also complicated, since the tablets are very sensitive to air humidity. The stability of the tablets can be reduced drastically if in contact with air and humidity. Therefore an aluminium blister is the logical choice for packaging. This, however, demands also a high temperature (190 °C for 1/10 of a second), which might damage the bacteria in the tablet. Further on, if the pressure during tablet forming is low, the tablet is soft and it will disintegrate easily. Thus, it will not be possible to press the tablet through the aluminium blister without breaking the tablet into pieces. This lowers the commercial value of the tablets and is undesirable from the marketing point of view.

Consequently, there is a need for providing a process suitable for the manufacture of lactic acid bacteria tablets having an effective amount of viable bacteria. The present invention aims at satisfying that need. The invention is defined in the appended claims, the disclosure of which is incorporated herein by reference.

The invention relates to a process for producing lactic acid bacteria tablets wherein a mixture of lactic acid bacteria and carrier comprising lactic acid bacteria approximately 7 x 10^9 CFU/g is formed and tablets are formed from the mass under pressure 25 N to 40 N, preferably 30 N to 35 N, to enable the substantial amount of bacteria to stay viable and to make the tablets hard enough to withstand pressing through aluminium blister of the package; the tablets are packaged in aluminium blisters right after the formation of the tablets.

In a preferred embodiment of the invention the amount of bacteria in the mass is 25 to 35 %. The size of the tablets is preferably 0.5-0.75 g. The amount of viable bacteria in the tablet according to the invention is at the least 1 x 10^9 CFU/g, preferably over 3 x 10^9 CFU/g. A preferred form of the tablets is round.

In a preferred embodiment of the invention the lactic acid bacteria are aerobic or facultative aerobic. All ingredients have preferably relative humidity less than 4 degrees and all ingredients are at the same temperature.

In a preferred embodiment the aluminium blister's folio size is 15 to 25 microns, preferably 20 microns. The packaging is preferably done under N_2.

The invention will now be described in more detail. The terms used in the specification and in the claims have the meaning usually understood in microbiology, biochemistry, tabletting and related technology.

The invention according to the application relates to a process for producing lactic acid bacteria tablets containing a physiologically effective amount of lactic acid bacteria which tablets are packaged properly for storage and transportation. Aerobic lactic acid bacteria are preferred since they are not sensitive to air and oxygen. Thus, no special conditions are needed during production and storage. Anaerobic lactic acid bacteria can be used if the manufacturing conditions are suitable, especially if the lactic acid bacteria are properly lyophylized. Tablets can be formed in such a production method that the bacteria are kept viable during the tabletting and storing and so that the tablets are packaged properly for storage and transportation.

Experimentation has showed that about 30 to 70 % of the bacteria will generally be lost in the tabletting process according to the invention. The initial mass should contain such an amount of the bacteria that a physiologically sufficient amount remains alive during the production. The mass used according to the present invention comprises 25 – 35 % lactic acid bacteria and the rest carrier, said mass initially containing 6 to 8, preferably about 7 x 10^9 CFU/g bacteria. Thus, the mass provides a sufficient amount of viable bacteria to the final product without raising the costs too high. The amount of viable bacteria in the product is considerably larger than in the tablets produced by the prior art.

Any lactic acid bacteria can be used in the process according to the invention. In a preferred embodiment the lactic acid bacteria chosen are all aerobic since they are air and oxygen resistant. They stay viable in normal manufacturing and storage conditions.

The carrier used in the present invention can be any conventional carrier used in the tabletting, e.g. inulin, sorbitol, cellulose.

Tablets can be produced in various shapes according to the invention. In a preferred embodiment the tablets have a round form, since the temperature of the process is then eliminated more quickly and the temperature is evenly dispersed. This ensures that more bacteria will survive the process than when using other tablet forms.

The pressure used in the process according to the invention is lower than in conventional processes. In a preferred embodiment the pressure during tabletting is between 30 N and 35 N which is low enough to protect the bacteria from dying and high enough to make the tablet strong enough to withstand pressing through the bottom of the aluminium plate of a blister without damages.

Conventional tableting machines can be used for making the tablets according to this invention. However, special apparatus especially designed for the process according to the invention can be made if production volumes are very high.

In the process according to the invention the aluminium blister's folio size is thinner than usually. In a preferred embodiment of the invention the aluminium blister's folio size is 20

microns. This is thin enough to allow the tablets to be easily pressed through the blister. The blister is, however, thick enough to protect the tablets during storage and transportation.

Moisture and temperature gradients can cause unevenness in the process and deterioration of quality. All ingredients have preferably less than 4 degrees of relative humidity and they should be at the same temperature during the process to avoid humidity and condensation. Humidity and condensation easily enhance molding in tablets as well as disintegration.

In the process according to this invention mass is produced of lactic acid bacteria and a carrier by conventional mixing methods. Once the mass is done, at approximately 7×10^9 CFU/g (bacteria and carrier) tabletting should follow almost immediately or as soon as possible. This prevents the bacteria from starting to ferment and stops any unwanted micro-organisms from contaminating the mass.

In a preferred embodiment of the invention, the tablets are formed and then they are packaged in blisters as soon as their temperature is the same as the one of the packaging room to avoid humidity and condensation. The folio has preferably a large surface and few tablets, so that the tablets are fairly far from each other to avoid concentration of temperature, which might kill lactic acid bacteria. If possible the packaging is done under N_2 for further stability.

In a preferred embodiment of the invention the tablets are large to avoid too much bacteria to be lost during the process but yet they must be small enough to be orally administered (0.5-0.75 g). In large tablets the proportion of the individual bacteria that are exposed to high temperature is lower than in smaller tablets.

A further advantage of the invention is that lactic acid bacteria tablets are practically packaged for transportation. They are easily absorbed, no water is needed and they are reactive with mouth enzymes. This makes it easy to administer the tablets even in conditions with poor hygiene or nutrition.

Claims

1. A process for producing lactic acid bacteria tablets **characterized** in that a mass is formed by mixing lactic acid bacteria and carrier, said mass comprising lactic acid bacteria at least approximately 7 x 10^9 CFU/g; tablets are formed from said mass under a pressure of 25 N to 40 N, preferably 30 N to 35 N to enable a substantial amount of the bacteria to remain viable and to make the tablets hard enough to withstand pressing through an aluminium blister of the package; the tablets are packaged in aluminium blisters.

2. A process according to claim 1 **characterized** in that the amount of bacteria in the mass is 25 to 35 % of the total weight of the mass.

3. A process according to claim 1 or 2 **characterized** in that the weight of the tablets is 0.5-0.75 g.

4. A process according any one of the claims 1 to 3 **characterized** in that the amount of viable bacteria in the tablet is at the least 1 x 10^9 CFU/g, preferably over 3 x 10^9 CFU/g.

5. A process according to any one of the claims 1 to 4 **characterized** in that said tablets are round.

6. A process according to any one of the claims 1 to 5 **characterized** in that said lactic acid bacteria are aerobic or facultative aerobic bacteria.

7. A process according to any one of the claims 1 to 6 **characterized** in that all of said ingredients have a relative humidity less than 4 degrees and that all ingredients are at substantially the same temperature at mixing.

8. A process according to any one of the claims 1 to 7 **characterized** in that said aluminium blister's folio size is 15 to 25 microns, preferably 20 microns.

9. A process according to any one of the claims 1 to 8 **characterized** in that the packaging is done under N_2.

The invention is now illustrated in embodiments that do not limit the use of the invention. A person skilled in the art will find other ways to use the invention.

Example 1

30 % lactic acid bacteria and 70 % carrier having a mixture of inulin, sorbitol and cellulose, were mixed to form a mass containing lactic acid bacteria approximately 7 x 10^9 CFU/g. Once the mass was ready, it was immediately pressed into tablets using a pressure between 30 and 35 N. The temperature used was 90 °C. Subsequently the tablets were allowed to cool. When the temperature of the tablets was equal to the temperature of the packaging room, the tablets were packaged into aluminium blisters. The aluminium blister's folio size used was 20 microns.

In order to measure the amount of the viable cells in the tablets the tablets were soaked in water for 24 hours. The amount of viable cells was measured and the bacteria were found to have a residual activity well above 1 x 10^9 CFU/g.

Example 2

6.5 kg of pectinase (3.5 % of the initial mass) and 45 kg of lactic acid bacteria plus ca. 5 kg of *Enterococcus faecium* in stock was mixed to a mass containing 20 % inulin, 18.5 % sorbitol, 32 % cellulose and 1 % magnesium stearate. Tablets were formed having a weight of 500 mg and a diameter of 12 mm. The tablets were packaged in aluminium blisters, 20 tablets in each blister. The size of the blister was 7.5 cm x 10 cm and the distance between the tablets was 0.5 cm.

Example 3

Tablets were produced both using a conventional tabletting method with a pressure of 50 N and a method according to the invention with a pressure of 30 N. The amounts of viable bacteria were measured from the powder used for tabletting and from the tablets. The amount of bacteria in the powder was 2.9 x 10^9 CFU/g and the amount in the conventional tablet was 5.0 x 10^7 CFU/g and in the tablet according to the invention 5 x 10^9 CFU/g, which is 17 % and 28 % of the amount of bacteria in the powder, respectively.

INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K35/74

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
PAJ, EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 07822 A (DETUM AB) 6 March 1997 (1997-03-06) page 4, line 8 -page 5, line 28 ---	1-9
P,A	WO 01 37880 A (WASA MEDICALS AB) 31 May 2001 (2001-05-31) the whole document ---	1-9
A	EP 0 955 058 A (MORINAGA MILK INDUSTRY CO LTD) 10 November 1999 (1999-11-10) the whole document ---	1-9
A	EP 0 599 479 A (PASTEUR INST KYOTO) 1 June 1994 (1994-06-01) the whole document -----	1-9

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9707822 A	06-03-1997	SE 513815 C2	06-11-2000
		AU 705507 B2	27-05-1999
		AU 6893196 A	19-03-1997
		BR 9610015 A	06-07-1999
		CA 2230238 A1	06-03-1997
		CN 1200038 A	25-11-1998
		EP 0845999 A1	10-06-1998
		JP 11511458 T	05-10-1999
		NO 980777 A	24-02-1998
		PL 325061 A1	06-07-1998
		SE 9502941 A	26-02-1997
		WO 9707822 A1	06-03-1997
WO 0137880 A	31-05-2001	SE 515310 C2	09-07-2001
		AU 1567301 A	04-06-2001
		SE 0001447 A	26-05-2001
		WO 0137880 A1	31-05-2001
EP 0955058 A	10-11-1999	EP 0955058 A1	10-11-1999
		WO 9830235 A1	16-07-1998
EP 0599479 A	01-06-1994	JP 2051579 C	10-05-1996
		JP 6206826 A	26-07-1994
		JP 7055908 B	14-06-1995
		EP 0599479 A2	01-06-1994
		US 5556785 A	17-09-1996
		US 5662900 A	02-09-1997